# IL-8 RECEPTOR ANTAGONISTS

## FIELD OF THE INVENTION

This invention relates to novel sulfonamide substituted diphenyl urea compounds, pharmaceutical compositions, processes for their preparation, and use thereof in treating IL-8, GROα, GROβ, GROγ, NAP-2, and ENA-78 mediated diseases.

## BACKGROUND OF THE INVENTION

Many different names have been applied to Interleukin-8 (IL-8), such as neutrophil attractant/activation protein-1 (NAP-1), monocyte derived neutrophil chemotactic factor (MDNCF), neutrophil activating factor (NAF), and T-cell lymphocyte chemotactic factor. Interleukin-8 is a chemoattractant for neutrophils, basophils, and a subset of T-cells. It is produced by a majority of nucleated cells including macrophages, fibroblasts, endothelial and epithelial cells exposed to TNF, IL-1α, IL-1β or LPS, and by neutrophils themselves when exposed to LPS or chemotactic factors such as FMLP. M. Baggiolini et al., J. Clin. Invest. *84*, 1045 (1989); J. Schroder et al, J. Immunol. *139*, 3474 (1987) and J. Immunol. *144*, 2223 (1990) ; Strieter, et al., Science *243*, 1467 (1989) and J. Biol. Chem. *264*, 10621 (1989); Cassatella et al., J. Immunol. *148*, 3216 (1992).

GROα, GROβ, GROγ and NAP-2 also belong to the chemokine family. Like IL-8 these chemokines have also been referred to by different names. For instance GROα, β, γ have been referred to as MGSAα, β and γ respectively (Melanoma Growth Stimulating Activity), see Richmond et al., J. Cell Physiology 129, 375 (1986) and Chang et al., J. Immunol 148, 451 (1992). All of the chemokines of the α-family which possess the ELR motif directly preceding the CXC motif bind to the IL-8 B receptor (CXCR2).

IL-8, GROα, GROβ, GROγ, NAP-2, and ENA-78 stimulate a number of functions in vitro. They have all been shown to have chemoattractant properties for neutrophils, while IL-8 and GROα have demonstrated T-lymphocytes, and basophilic chemotactic activity. In addition IL-8 can induce histamine release from basophils from both normal and atopic individuals. GRO-α and IL-8 can in addition, induce lysozomal enzyme release and respiratory burst from neutrophils. IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on neutrophils

without de novo protein synthesis. This may contribute to increased adhesion of the neutrophils to vascular endothelial cells. Many known diseases are characterized by massive neutrophil infiltration. As IL-8, GROα, GROβ, GROγ and NAP-2 promote the accumulation and activation of neutrophils, these chemokines have been implicated in a wide range of acute and chronic inflammatory disorders including psoriasis and rheumatoid arthritis, Baggiolini et al., FEBS Lett. *307*, 97 (1992); Miller et al., Crit. Rev. Immunol. *12*, 17 (1992); Oppenheim et al., Annu. Rev. Immunol. *9*, 617 (1991); Seitz et al., J. Clin. Invest. *87*, 463 (1991); Miller et al., Am. Rev. Respir. Dis. *146*, 427 (1992); Donnely et al., Lancet *341*, 643 (1993). In addition the ELR chemokines (those containing the amino acids ELR motif just prior to the CXC motif) have also been implicated in angiostasis, Strieter et al., Science 258, 1798 (1992).

In vitro, IL-8, GROα, GROβ, GROγ and NAP-2 induce neutrophil shape change, chemotaxis, granule release, and respiratory burst, by binding to and activating receptors of the seven-transmembrane, G-protein-linked family, in particular by binding to IL-8 receptors, most notably the IL-8b receptor (CXCR2). Thomas et al., J. Biol. Chem. *266*, 14839 (1991); and Holmes et al., Science *253*, 1278 (1991). The development of non-peptide small molecule antagonists for members of this receptor family has precedent. For a review see R. Freidinger in: Progress in Drug Research, Vol. 40, pp. 33-98, Birkhauser Verlag, Basel 1993. Hence, the IL-8 receptor represents a promising target for the development of novel anti-inflammatory agents.

Two high affinity human IL-8 receptors (77% homology) have been characterized: IL-8Ra, which binds only IL-8 with high affinity, and IL-8Rb, which has high affinity for IL-8 as well as for GROα, GROβ, GROγ and NAP-2. See Holmes et al., supra; Murphy et al., Science *253*, 1280 (1991); Lee et al., J. Biol. Chem. *267*, 16283 (1992); LaRosa et al., J. Biol. Chem. *267*, 25402 (1992); and Gayle et al., J. Biol. Chem. *268*, 7283 (1993).

There remains a need for treatment, in this field, for compounds, which are capable of binding to the IL-8 a or b receptor. Therefore, conditions associated with an increase in IL-8 production (which is responsible for chemotaxis of neutrophil and T-cells subsets into the inflammatory site) would benefit by compounds, which are inhibitors of IL-8 receptor binding.

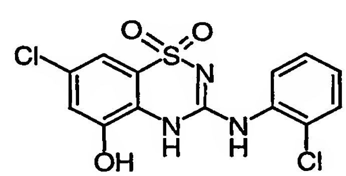
## SUMMARY OF THE INVENTION

This invention provides for a method of treating a chemokine mediated disease, wherein the chemokine is one which binds to an IL-8 a or b receptor and which method comprises administering an effective amount of a compound selected from the following group of substituted 3-phenylamino-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxides :

7-chloro-3-[(2-chlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
7-chloro-3-(cyclopentylamino)-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
7-chloro-3-[(2,3-dichlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
3-[(2-chlorophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
3-[(2-bromophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
7-nitro-3-{[2-(phenyloxy)phenyl]amino}-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
3-[(2-chloro-3-fluorophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide
*N*-(2-chlorophenyl)-5-(methyloxy)-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide
and pharmaceutically acceptable salts thereof

## SYNTHETIC EXAMPLES

Example 1



7-chloro-3-[(2-chlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

1) 4-Chloro-2-(methyloxy)aniline

A suspension of 2-nitro-5-chloroanisole(7.00g), zinc dust(12.5g) and acetic acid (7.8ml) in ethanol(100ml) was refluxed for 30-60mins. The suspension was then filtered through celite and washed with ethyl acetate. The filtrate was concentrated to give a gray solid, which was redissolved in ethyl acetate(250ml) and washed with water, saturated aqueous sodium bicarbonate solution. The organic phase was dried

over $Na_2SO_4$. The solvent was evaporated to give the title compound as a brown oil (4.65g, 80%). LC/MS: m/z 158 (M+H).

1b) <u>7-chloro-5-(methyloxy)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide</u>

A solution of 4-Chloro-2-(methyloxy)aniline (4.65g) in nitropropane(5mL) was added to a solution of chlorosulfonyl isocyanate(3.16mL) in nitropropane(45mL) at -40°C for 5mins. The reaction mixture was allowed to warm up to 0°C. Aluminum chloride (4.94g) was added at once, resulting in a clear solution. The reaction mixture was heated at 110 °C for 20mins. After cooling down to room temperature, the reaction mixture was poured on ice-water mixture. The precipitate was filtered off and washed with water, dried in *vacuo* to give brown solid (4.0g, 52%). LC/MS: m/z 263 (M+H).

1c) <u>7-chloro-*N*-(2-chlorophenyl)-5-(methyloxy)-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide</u>

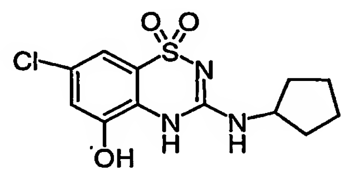
Triflic anhydride(1.19ml) was added to a solution of 7-chloro-5-(methyloxy)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (740mg) and pyridine(0.82ml) in dichloromethane(15ml) at –78 °C. The reaction mixture was stirred for 1.5hrs at this temperature before quenching with aqueous ammonium chloride(5ml). After warming up to room temperature, the aqueous phase was extracted with methylene chloride(3X15ml). The combined organic phase was dried over $MgSO_4$ and concentrated to give the amidoyl triflate(0.86g, 56%).

The resulting amidoyl triflate(250mg) was dissolved in methylene chloride(15ml). 2-Chloroaniline(0.20ml) was added at -78 °C. The solution was allowed to warm up to room temperature overnight. The reaction mixture was diluted with dichloromethane (40ml) and washed with saturated aqueous sodium bicarbonate(50ml). The aqueous phase was extracted with dichloromethane(2X40ml). The combined organic extracts were dried over $MgSO_4$ and evaporated in *vacuo*. The residue was dissolved in DMSO(1.5ml) and purified by Gilson preparatory HPLC to give the title compound(156mg, 63%). LC/MS: m/z 372 (M+H).

1d) 7-chloro-3-[(2-chlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

7-chloro-*N*-(2-chlorophenyl)-5-(methyloxy)-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide (130mg) was dissolved in methylenechloride(10ml). $BBr_3$(1ml) was added. The reaction mixture was refluxed for 6 hr. LCMS showed that there was no starting material left. The reaction mixture was purified by Gilson preparatory HPLC to give the title compound(91mg, 72%). LC/MS: m/z 358 (M+H).

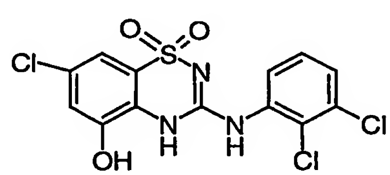
Example 2



7-chloro-3-(cyclopentylamino)-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 1c) and 1d), 7-chloro-5-(methyloxy)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (250mg) was treated with cyclopentylamine (163mg, 1.91mmol) followed by removal of protecting group with tribromoborane to give the title product 122mg (60%). $^1$H NMR ($CD_3OD$) δ: 7.15(1H, s), 6.93 (1H, s), 4.21(1H, m), 2.08(2H, m), 1.72(4H, m), 1.53(2H,m).

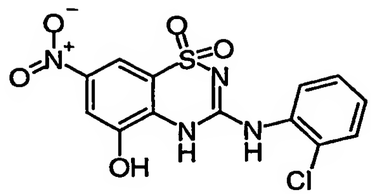
Example 3



7-chloro-3-[(2,3-dichlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 1c) and 1d), 7-chloro-5-(methyloxy)-4*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (740mg, 2.82mmol) was treated with triflic anhydride (1.991g, 7.06mmol) to give the crude amidoyl triflate 0.86g. One portion of this crude material (316mg, 0.81mmol) was then reacted with 2,3-dichloroanaline (260mg, 1.62mmol) followed by removal of protecting group with tribromoboron to yield the title prouduct 130mg (41%). LC/MS: m/z 393 (M+H).

Example 4



3-[(2-chlorophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

4a) 5-(methyloxy)-7-nitro-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide

Followed the general procedure outlined in Example 1b), 2-methyloxy-4-nitroaniline (4.97g) was treated with chlorosulfonyl isocyanate(3.17ml) in nitroethane(45ml) at –10 °C over 10mins. The *in situ* cyclization with aluminum chloride (5g) yielded the desired product (4.9g, 60%). LC/MS: m/z 274 (M+H).

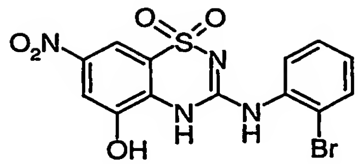
4b) *N*-(2-chlorophenyl)-5-(methyloxy)-7-nitro-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide

Followed the general procedure outlined in Example 1c), 5-(methyloxy)-7-nitro-4*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (70mg) was treated with triflic anhydride (0.13mL) followed by reacting with 2-Chloroaniline(0.034ml) to give the title compound(33mg, 33%). $^1$H NMR ($CD_3OD$) δ: 8.27(1H, s), 8.16 (1H, d), 7.99(1H, s), 7.49(1H, d), 7.36(1H, dd), 7.18(1H, dd), 4.17 (3H, s).

4c) 3-[(2-chlorophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

*N*-(2-chlorophenyl)-5-(methyloxy)-7-nitro-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide (120mg) was dissolved in DMSO(3ml). LiCl (60mg) was added. The reaction mixture was heated at 150 °C for 8 hr. LCMC showed that there was no starting meterial left. After cooling down to room temperature, LiCl was filtered off. The filtrate was purified by Gilson preparatory HPLC to give the title compound(92mg, 80%). LC/MS: m/z 383 (M+H).

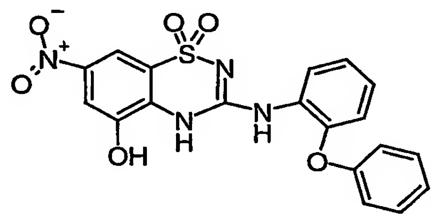
Example 5



3-[(2-bromophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 1c) and 4c), 5-(methyloxy)-7-nitro-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (100mg, 0.36mmol) was treated with 2-bromoaniline (188mg, 1.09mmol) followed by removal of protecting group with LiCl to give the title prouduct 22mg (15%). $^1$H NMR (DMSO) δ: 9.31(1H, s), 7.93(1H, s), 7.77(2H, m), 7.71(1H, d), 7.44(1H, t), 7.21(1H, t).

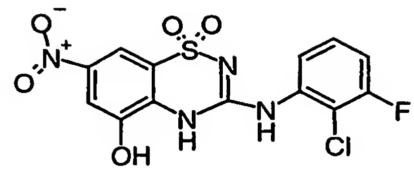
Example 6



7-nitro-3-{[2-(phenyloxy)phenyl]amino}-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 1c) and 4c), 5-(methyloxy)-7-nitro-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (623mg, 2.27mmol) was treated with 2-phenoxyaniline (842mg, 4.54mmol) followed by removal of protecting group with LiCl to give the title prouduct 122mg (13%). $^1$H NMR (DMSO) δ: 9.68(1H, s), 8.16(1H, d), 7.93(1H, s), 7.81(1H, br), 7.41(1H, t), 7.26-7.09(3H,m), 7.08(2H,d), 6.86(1H, d).

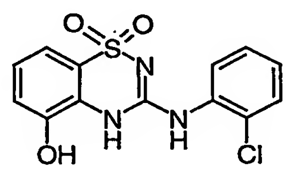
Example 7



3-[(2-chloro-3-fluorophenyl)amino]-7-nitro-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 1c) and 4c), 5-(methyloxy)-7-nitro-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (500mg, 1.82mmol) was treated with 2-chloro-3-fluoro-aniline (532mg, 3.64mmol) followed by removal of protecting group with LiCl to give the title prouduct 250mg (35%). $^1$H NMR ($CD_3OD$) δ: 8.04(1H, s), 7.87(1H, d), 7.71(1H, s), 7.24(1H, m), 7.03(1H, m), 2.86(1H,s).

Example 8



3-[(2-chlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

8a) *N*-(2-chlorophenyl)-5-(methyloxy)-4*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide

Followed the general procedure outlined in Example 1b) and 1c), 2-methyloxyaniline (36.4g, 29.55mmol) was treated with chlorosulfonyl isocyanate (5.16g, 36.45mmol) and trichloroaluminum (5g, 37.44mmol) to give the crude 5-(methyloxy)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide, which then reacted with 2-chloroaniline to yield the title product 3.76g (38%). $^1$H NMR ($CD_3OD$) δ: 8.13(1H, d), 7.43 (1H, d), 7.32(4H, m), 7.11(1H, t), 4.08(3H, s).

8b) 3-[(2-chlorophenyl)amino]-4*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxide

Followed the general procedure outlined in Example 4c), *N*-(2-chlorophenyl)-5-(methyloxy)-2*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxide (0.5g, 1.48mmol) was treated with lithium chloride (0.5g, 11.8mmol) to give the title compound 247mg (52%). $^1$H NMR ($CD_3OD$) δ: 8.13(1H, d), 7.43 (1H, d), 7.32(1H, t), 7.21(1H, d), 7.17(2H, t), 7.06(1H,d).

## METHOD OF TREATMENT

The compounds of Formula (I), or a pharmaceutically acceptable salt thereof can be used in the manufacture of a medicine for the prophylactic or therapeutic treatment of any disease state in a human, or other mammal, which is exacerbated or caused by excessive or unregulated IL-8 cytokine production by such mammal's cell, such as but not limited to monocytes and/or macrophages, or other chemokines which bind to the IL-8 a or b receptor, also referred to as the type I or type II receptor.

Accordingly, the present invention provides a method of treating a chemokine mediated disease, wherein the chemokine is one which binds to an IL-8 α or β receptor and which method comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In particular, the chemokines are IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78.

The compounds of Formula (I) are administered in an amount sufficient to inhibit cytokine function, in particular IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78, such that they are biologically regulated down to normal levels of physiological function, or in some case to subnormal levels, so as to ameliorate the disease state. Abnormal levels of IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 for instance in the context of the present invention, constitute: (i) levels of free IL-8 greater than or equal to 1 picogram per mL; (ii) any cell associated IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 above normal physiological levels; or (iii) the presence of IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 above basal levels in cells or tissues in which IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 respectively, is produced.

The compounds of Formula (I), in generally have been shown to have a longer $t_{1/2}$ and improved oral bioavailabilty over the compounds disclosed in WO 96/25157 and WO 97/29743 whose disclosures are incorporated herein by reference.

There are many disease states in which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. . Chemokine mediated diseases include psoriasis, atopic dermatitis, arthritis (either osteo- or rheumatoid), asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome, inflammatory bowel disease, Crohn's disease, ulcerative colitis, stroke, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, cardiac and renal reperfusion injury, glomerulonephritis, thrombosis, graft vs. host reaction, alzheimers disease, allograft rejections, malaria, restinosis, angiogenesis, atherosclerosis, osteoporosis, gingivitis, viral diseases such as rhinovirus or undesired hematopoietic stem cell release.

These diseases are primarily characterized by massive neutrophil infiltration, T-cell infiltration, or neovascular growth, and are associated with increased IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 production which is responsible for the chemotaxis of neutrophils into the inflammatory site or the directional growth of endothelial cells. In contrast to other inflammatory cytokines (IL-1, TNF, and IL-6), IL-8, GROα, GROβ, GROγ, NAP-2 or ENA-78 have the unique property of promoting neutrophil chemotaxis, enzyme release including but not limited to elastase release as well as superoxide production and activation. The α-chemokines but particularly, GROα, GROβ, GROγ, NAP-2 or ENA-78, working through the IL-8 type I or II receptor can promote the neovascularization of tumors by promoting the directional growth of endothelial cells. Therefore, the inhibition of IL-8 induced chemotaxis or activation would lead to a direct reduction in the neutrophil infiltration.

Recent evidence also implicates the role of chemokines in the treatment of HIV infections, Littleman *et al.*, Nature 381, pp. 661 (1996) and Koup *et al.*, Nature 381, pp. 667 (1996).

Present evidence also indicates the use of IL-8 inhibitors in the treatment of atherosclerosis. The first reference, Boisvert et al., J. Clin. Invest, 1998, 101:353-363 shows, through bone marrow transplantation, that the absence of IL-8 receptors on stem

cells (and, therefore, on monocytes/macrophages) leads to a reduction in the development of atherosclerotic plaques in LDL receptor deficient mice. Additional supporting references are: Apostolopoulos, et al., Arterioscler. Thromb. Vasc. Biol. 1996, 16:1007-1012; Liu, et al., Arterioscler. Thromb. Vasc. Biol, 1997, 17:317-323; Rus, et al., Atherosclerosis. 1996, 127:263-271.; Wang et al., J. Biol. Chem. 1996, 271:8837-8842; Yue, et al., Eur. J. Pharmacol. 1993, 240:81-84; Koch, et al., Am. J. Pathol., 1993, 142:1423-1431.; Lee, et al., Immunol. Lett., 1996, 53, 109-113.; and Terkeltaub et al., Arterioscler. Thromb., 1994, 14:47-53.

The present invention also provides for a means of treating, in an acute setting, as well as preventing, in those individuals deemed susceptible to, CNS injuries by the chemokine receptor antagonist compounds of Formula (I).

CNS injuries as defined herein include both open or penetrating head trauma, such as by surgery, or a closed head trauma injury, such as by an injury to the head region. Also included within this definition is ischemic stroke, particularly to the brain area.

Ischemic stroke may be defined as a focal neurologic disorder that results from insufficient blood supply to a particular brain area, usually as a consequence of an embolus, thrombi, or local atheromatous closure of the blood vessel. The role of inflammatory cytokines in this area has been emerging and the present invention provides a mean for the potential treatment of these injuries. Relatively little treatment, for an acute injury such as these has been available.

TNF-α is a cytokine with proinflammatory actions, including endothelial leukocyte adhesion molecule expression. Leukocytes infiltrate into ischemic brain lesions and hence compounds which inhibit or decrease levels of TNF would be useful for treatment of ischemic brain injury. See Liu *et al.*, Stroke, Vol. 25., No. 7, pp. 1481-88 (1994) whose disclosure is incorporated herein by reference.

Models of closed head injuries and treatment with mixed 5-LO/CO agents is discussed in Shohami *et al.*, J. of Vaisc & Clinical Physiology and Pharmacology, Vol. 3, No. 2, pp. 99-107 (1992) whose disclosure is incorporated herein by reference. Treatment which reduced edema formation was found to improve functional outcome in those animals treated.

The compounds of Formula (I) are administered in an amount sufficient to inhibit IL-8, binding to the IL-8 a or b receptors, from binding to these receptors, such as evidenced by a reduction in neutrophil chemotaxis and activation. The discovery that the compounds of Formula (I) are inhibitors of IL-8 binding is based upon the effects of the compounds of Formulas (I) in the *in vitro* receptor binding assays which are described herein. The compounds of Formula (I) have been shown to be inhibitors of type II IL-8 receptors.

As used herein, the term "IL-8 mediated disease or disease state" refers to any and all disease states in which IL-8, GRO$\alpha$, GRO$\beta$, GRO$\gamma$, NAP-2 or ENA-78 plays a role, either by production of IL-8, GRO$\alpha$, GRO$\beta$, GRO$\gamma$, NAP-2 or ENA-78 themselves, or by IL-8, GRO$\alpha$, GRO$\beta$, GRO$\gamma$, NAP-2 or ENA-78 causing another monokine to be released, such as but not limited to IL-1, IL-6 or TNF. A disease state in which, for instance, IL-1 is a major component, and whose production or action, is exacerbated or secreted in response to IL-8, would therefore be considered a disease state mediated by IL-8.

As used herein, the term "chemokine mediated disease or disease state" refers to any and all disease states in which a chemokine which binds to an IL-8 a or □ receptor plays a role, such as but not limited to IL-8, GRO-$\alpha$, GRO-$\beta$, GRO$\gamma$, NAP-2or ENA-78. This would include a disease state in which, IL-8 plays a role, either by production of IL-8 itself, or by IL-8 causing another monokine to be released, such as but not limited to IL-1, IL-6 or TNF. A disease state in which, for instance, IL-1 is a major component, and whose production or action, is exacerbated or secreted in response to IL-8, would therefore be considered a disease stated mediated by IL-8.

As used herein, the term "cytokine" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a mononuclear cell, such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibroblasts, basophils, neutrophils, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes

and B-lymphocytes. Lymphokines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-$\alpha$) and Tumor Necrosis Factor beta (TNF-ß).

As used herein, the term "chemokine" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response, similar to the term "cytokine" above. A chemokine is primarily secreted through cell transmembranes and causes chemotaxis and activation of specific white blood cells and leukocytes, neutrophils, monocytes, macrophages, T-cells, B-cells, endothelial cells and smooth muscle cells. Examples of chemokines include, but are not limited to IL-8, GRO-$\alpha$, GRO-$\beta$, GRO-$\gamma$, NAP-2, ENA-78, IP-10, MIP-1$\alpha$, MIP-$\beta$, PF4, and MCP 1, 2, and 3.

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of Formula (I) and a pharmaceutically acceptable carrier or diluent..

Compounds of Formula (I), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of Formula (I) may be administered in conventional dosage forms prepared by combining a compound of Formula (I) with standard pharmaceutical carriers according to conventional procedures. The compounds of Formula (I) may also be administered in conventional dosages in combination with a known, second therapeutically active compound. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in

the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of Formula (I) may be administered topically, that is by non-systemic administration. This includes the application of a compound of Formula (I) externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, for instance from 1% to 2% by weight of the Formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the Formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an

agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100$^{\circ}$C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Compounds of formula (I) may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such

administration may be prepared by conventional techniques. Compounds of Formula (I) may also be administered by inhalation, that is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

For all methods of use disclosed herein for the compounds of Formula (I) the daily oral dosage regimen will preferably be from about 0.01 to about 80 mg/kg of total body weight. The daily parenteral dosage regimen about 0.001 to about 80 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 mg to 150 mg, administered one to four, preferably two or three times daily. The daily inhalation dosage regimen will preferably be from about 0.01 mg/kg to about 1 mg/kg per day. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of Formula (I) or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of Formula (I) or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The invention will now be described by reference to the following biological examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

## BIOLOGICAL EXAMPLES

The IL-8, and GRO-α chemokine inhibitory effects of compounds of the present invention are determined by the following *in vitro* assay:

### Receptor Binding Assays:

[$^{125}$I] IL-8 (human recombinant) is obtained from Amersham Corp., Arlington Heights, IL, with specific activity 2000 Ci/mmol. GRO-α is obtained from NEN- New England Nuclear. All other chemicals are of analytical grade. High levels of

recombinant human IL-8 type a and b receptors were individually expressed in Chinese hamster ovary cells as described previously (Holmes, *et al.*, Science, **1991**, *253*, 1278). The Chinese hamster ovary membranes were homogenized according to a previously described protocol (Haour, *et al.*, J. Biol. Chem., 249 pp 2195-2205 (1974)). Except that the homogenization buffer is changed to 10mM Tris-HCL, 1mM $MgSO_4$, 0.5mM EDTA (ethylene-diaminetetra-acetic acid), 1mM PMSF (α-toluenesulphonyl fluoride), 0.5 mg/L Leupeptin, pH 7.5. Membrane protein concentration is determined using Pierce Co. micro-assay kit using bovine serum albumin as a standard. All assays are performed in a 96-well micro plate format. Each reaction mixture contains $^{125}I$ IL-8 (0.25 nM) or $^{125}I$ GRO-α and 0.5 μg/mL of IL-8Ra or 1.0 μg/mL of IL-8Rb membranes in 20 mM Bis-Trispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM $MgSO_4$, 0.1 mM EDTA, 25 mM Na and 0.03% CHAPS. In addition, drug or compound of interest is added which has been pre-dissolved in DMSO so as to reach a final concentration of between 0.01nM and 100 uM. The assay is initiated by addition of $^{125}I$-IL-8. After 1 hour at room temperature the plate is harvested using a Tomtec 96-well harvester onto a glass fiber filtermat blocked with 1% polyethylenimine/ 0.5% BSA and washed 3 times with 25 mM NaCl, 10 mM TrisHCl, 1 mM $MgSO_4$, 0.5 mM EDTA, 0.03 % CHAPS, pH 7.4. The filter is then dried and counted on the Betaplate liquid scintillation counter. The recombinant IL-8 Ra, or Type I, receptor is also referred to herein as the non-permissive receptor and the recombinant IL-8 Rb, or Type II, receptor is referred to as the permissive receptor.

Representative compounds of Formula (I), Examples 1 to 106 have exhibited positive inhibitory activity in this assay at $IC_{50}$ levels < 30 uM.

**Chemotaxis Assay :**

The *in vitro* inhibitory properties of these compounds are determined in the neutrophil chemotaxis assay as described in Current Protocols in Immunology, vol. I, Suppl 1, Unit 6.12.3., whose disclosure is incorporated herein by reference in its entirety. Neutrophils where isolated from human blood as described in Current Protocols in Immunology Vol. I, Suppl 1 Unit 7.23.1, whose disclosure is incorporated

herein by reference in its entirety. The chemoattractants IL-8, GRO-α, GRO-β, GRO-γ and NAP-2 are placed in the bottom chamber of a 48 multiwell chamber (Neuro Probe, Cabin John, MD) at a concentration between 0.1 and 100 nM. The two chambers are separated by a 5 uM polycarbonate filter. When compounds of this invention are tested, they are mixed with the cells (0.001 - 1000 nM) just prior to the addition of the cells to the upper chamber. Incubation is allowed to proceed for between about 45 and 90 min at about 37°C in a humidified incubator with 5% $CO_2$. At the end of the incubation period, the polycarbonate membrane is removed and the top side washed, the membrane then stained using the Diff Quick staining protocol (Baxter Products, McGaw Park, IL, USA). Cells which have chemotaxed to the chemokine are visually counted using a microscope. Generally, four fields are counted for each sample, these numbers are averaged to give the average number of cells which had migrated. Each sample is tested in triplicate and each compound repeated at least four times. To certain cells (positive control cells) no compound is added, these cells represent the maximum chemotactic response of the cells. In the case where a negative control (unstimulated) is desired, no chemokine is added to the bottom chamber. The difference between the positive control and the negative control represents the chemotactic activity of the cells.

<u>**Elastase Release Assay:**</u>

The compounds of this invention are tested for their ability to prevent Elastase release from human neutrophils. Neutrophils are isolated from human blood as described in Current Protocols in Immunology Vol. I, Suppl 1 Unit 7.23.1. PMNs 0.88 x $10^6$ cells suspended in Ringer's Solution (NaCl 118, KCl 4.56, $NaHCO_3$ 25, $KH_2PO_4$ 1.03, Glucose 11.1, HEPES 5 mM, pH 7.4) are placed in each well of a 96 well plate in a volume of 50 ul. To this plate is added the test compound (0.001 - 1000 nM) in a volume of 50 ul, Cytochalasin B in a volume of 50 ul (20ug/ml) and Ringers buffer in a volume of 50 ul. These cells are allowed to warm (37 °C, 5% CO2, 95% RH) for 5 min before IL-8, GROα, GROβ, GROγ or NAP-2 at a final concentration of 0.01 - 1000 nM was added. The reaction is allowed to proceed for 45 min before the 96 well plate is centrifuged (800 xg 5 min.) and 100 ul of the supernatant removed. This supernatant is

added to a second 96 well plate followed by an artificial elastase substrate (MeOSuc-Ala-Ala-Pro-Val-AMC, Nova Biochem, La Jolla, CA) to a final concentration of 6 ug/ml dissolved in phosphate buffered saline. Immediately, the plate is placed in a fluorescent 96 well plate reader (Cytofluor 2350, Millipore, Bedford, MA) and data collected at 3 min intervals according to the method of Nakajima et al J. Biol. Chem. 254 4027 (1979). The amount of Elastase released from the PMNs is calculated by measuring the rate of MeOSuc-Ala-Ala-Pro-Val-AMC degradation.

**TNF-α in Traumatic Brain Injury Assay**

The present assay provides for examination of the expression of tumor necrosis factor mRNA in specific brain regions, which follow experimentally, induced lateral fluid-percussion traumatic brain injury (TBI) in rats. Adult Sprague-Dawley rats (n=42) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and subjected to lateral fluid-percussion brain injury of moderate severity (2.4 atm.) centered over the left temporaparietal cortex (n=18), or "sham" treatment (anesthesia and surgery without injury, n=18). Animals are sacrificed by decapitation at 1, 6 and 24 hr. post injury, brains removed, and tissue samples of left (injured) parietal cortex (LC), corresponding area in the contralateral right cortex (RC), cortex adjacent to injured parietal cortex (LA), corresponding adjacent area in the right cortex (RA), left hippocampus (LH) and right hippocampus (RH) are prepared. Total RNA are isolated and Northern blot hybridization is performed and quantitated relative to an TNF-α positive control RNA (macrophage = 100%). A marked increase of TNF- α mRNA expression is observed in LH (104±17% of positive control, $p < 0.05$ compared with sham), LC (105±21%, $p< 0.05$) and LA (69±8%, $p < 0.01$) in the traumatized hemisphere 1 hr. following injury. An increased TNF- α mRNA expression is also observed in LH (46±8%, $p < 0.05$), LC (30±3%, $p < 0.01$) and LA (32±3%, $p < 0.01$) at 6 hr which resolves by 24 hr following injury. In the contralateral hemisphere, expression of TNF- α mRNA is increased in RH (46±2%, $p < 0.01$), RC (4±3%) and RA (22±8%) at 1 hr and in RH (28±11%), RC (7±5%) and RA (26±6%, $p < 0.05$) at 6 hr but not at 24 hr following injury. In sham (surgery without injury) or naive animals, no consistent changes in expression of TNF- α mRNA are observed in any of the 6 brain areas in either hemisphere at any times. These results indicate that following parasagittal fluid-percussion brain injury, the temporal expression of TNF-α

mRNA is altered in specific brain regions, including those of the non-traumatized hemisphere. Since TNF-α is able to induce nerve growth factor (NGF) and stimulate the release of other cytokines from activated astrocytes, this post-traumatic alteration in gene expression of TNF-α plays an important role in both the acute and regenerative response to CNS trauma.

## CNS Injury model for IL-1β mRNA

This assay characterizes the regional expression of interleukin-1ß (IL-1ß) mRNA in specific brain regions following experimental lateral fluid-percussion traumatic brain injury (TBI) in rats. Adult Sprague-Dawley rats (n=42) are anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and subjected to lateral fluid-percussion brain injury of moderate severity (2.4 atm.) centered over the left temporaparietal cortex (n=18), or "sham" treatment (anesthesia and surgery without injury). Animals are sacrificed at 1, 6 and 24 hr. post injury, brains removed, and tissue samples of left (injured) parietal cortex (LC), corresponding area in the contralateral right cortex (RC), cortex adjacent to injured parietal cortex (LA), corresponding adjacent area in the right cortex (RA), left hippocampus (LH) and right hippocampus (RH) are prepared. Total RNA is isolated and Northern blot hybridization was performed and the quantity of brain tissue IL-1ß mRNA is presented as percent relative radioactivity of IL-1ß positive macrophage RNA which was loaded on the same gel. At 1 hr following brain injury, a marked and significant increase in expression of IL-1ß mRNA is observed in LC (20.0±0.7% of positive control, n=6, $p < 0.05$ compared with sham animal), LH (24.5±0.9%, $p < 0.05$) and LA (21.5±3.1%, $p < 0.05$) in the injured hemisphere, which remained elevated up to 6 hr. post injury in the LC (4.0±0.4%, n=6, $p < 0.05$) and LH (5.0±1.3%, $p < 0.05$). In sham or naive animals, no expression of IL-1ß mRNA is observed in any of the respective brain areas. These results indicate that following TBI, the temporal expression of IL-1ß mRNA is regionally stimulated in specific brain regions. These regional changes in cytokines, such as IL-1ß play a role in the post-traumatic.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual

publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.